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# Effect of Dimethyl Sulfoxide on Human Carcinoma Cells, Inhibition of Plasminogen Activator Synthesis, Change in Cell Morphology, and Alteration of Response to Cholera Toxin

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**Human carcinoma HEp-3 lost its tumorigenic and metastatic potential upon prolonged culture in vitro. This change was accompanied by a reduced production of plasminogen activator (PA) of the urokinase type (uPA), which is secreted by HEp-3 cells, a change in response to effectors that modulate uPA production, and an alteration of cell morphology. Similar but more rapid changes occurred when malignant HEp-3 cells were exposed to dimethyl sulfoxide (DMSO). uPA activity in the culture medium dropped below 50% of the control level within 6 h after the addition of DMSO and became undetectable after 24 h of treatment. This drop in uPA activity was not caused by an increased production of PA inhibitors. The cell-associated uPA decreased to 25 to 30% of the control level within 6 h of DMSO treatment and remained at this level for at least 96 h; the reduced uPA production was partially accounted for by a rapid decrease in the functional and chemical concentration of uPA mRNA. In contrast, the concentrations of most of the abundant mRNA species did not appear to be significantly affected, and cell growth was only slightly inhibited in the presence of DMSO. Malignant HEp-3 cells treated with DMSO responded to cholera toxin with an enhanced production of uPA, and their morphology became indistinguishable from that of nonmalignant HEp-3 cells grown in vitro for prolonged periods of time. All of the above changes were fully and rapidly reversible. The inhibitory effect of DMSO on PA production appears to be specific for uPA of human cell lines.**

Human epidermoid carcinoma HEp-3 cells, grown and passaged on the chorioallantoic membranes of chicken embryos, form large tumors at the site of inoculation and metastasize extensively into the organs of the embryo (10, 33). This malignant potential is retained only during propagation in vivo. After growth in cell culture for periods of several weeks to several months, the malignant potential of HEp-3 tumor-derived cells is gradually lost (34), although it can be reexpressed through prolonged exposure to in vivo conditions (35). Thus, the decline of the malignant potential accompanying continued growth in vitro represents a transition to a reversible phenotypic state, which is influenced by environmental factors (35).

One important correlate of malignancy is the ability of tumor cells to produce elevated levels of plasminogen activators (PAs), as demonstrated by the finding of enhanced PA production in virally and chemically transformed fibroblasts (37, 43, 46), high enzyme levels in experimental and human tumors (5, 6, 9, 14, 32, 38), and a coordinate modulation of tumor growth and PA production by hormones (4, 25, 28, 32). More directly, the metastasis of HEp-3 tumors is drastically reduced by an antibody which specifically inhibits the urokinase-type PA (uPA) secreted by the tumor cells (36). All of the above evidence strongly suggests that PAs play an important role in the expression of malignancy.

The conversion of malignant HEp-3 cells to a nonmalignant state, which takes place during culture in vitro, is accompanied by reduced uPA production, modified response to an adenylcyclase activator, and altered morphology (34). Since the conventional in vitro correlates of transformation, such as anchorage independence, reduced serum requirement, and increased cloning efficiency (42, 44), are not applicable to this cell line (34), we took advantage of those parameters which are characteristic of HEp-3 cells to further examine their phenotypic transition.

It has been shown in a number of systems that polar solvents, such as dimethyl sulfoxide (DMSO), can induce a variety of changes, including cell differentiation and reduced tumorigenicity (11, 15, 19, 21, 23, 27, 40, 41). We found that malignant HEp-3 cells, cultured in presence of DMSO, rapidly acquired characteristics similar to those associated with the nonmalignant phenotype. PA production was the first cellular property which was noticeably affected by DMSO treatment; we thus examined in more detail the mechanism of its inhibition by this compound. These results are discussed in the context of changes that accompany the conversion of HEp-3 cells from the malignant to the nonmalignant state.

## MATERIALS AND METHODS

Dulbecco modified Eagle medium was obtained from GIBCO, Grand Island, N.Y.; fetal bovine serum was obtained from Dutchland Laboratories Inc., Denver, Pa.; eggs were obtained from SPAFAS, Inc., Norwich, Conn.; Triton X-100, collagenase, Nonidet P-40, and DMSO were from Sigma Chemical Co., St. Louis, Mo.; proteinase K was obtained from Merck & Co., Inc., Rahway, N.J.; [$^{35}$S]methionine (specific activity, 400Ci/mmol) and En$^3$Hance were from New England Nuclear Corp., Boston, Mass.; RNase-free sucrose and cholera toxin were obtained from Schwarz-Mann, Division of Becton Dickinson and Co., Orangeburg, N.Y.; yeast tRNA was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; glutathione and oligo(dT)-cellulose type 77F were obtained from

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Pharmacia Fine Chemicals, Piscataway, N.J.; thymidine-3',5'-diphosphate was obtained from Calbiochem-Behring, La Jolla, Calif.; nitrocellulose (BA85) was obtained from Schleicher & Schuell, Inc., Keene, N.H.; and Trasylol was obtained from FBA Pharmaceuticals, New York, N.Y. Human plasminogen was purified from fresh human plasma as described previously (12). Fibrinogen (Calbiochem-Behring) was purified as described previously (45). Human urokinase standard was purchased from Leo Pharmaceuticals, Ballerup, Denmark. Highly purified uPA ($M_r$, 33,000) was a generous gift of Serono, Denens, Switzerland; the protein was labeled with $^{125}I$ as described previously (47). *Xenopus laevis* females were purchased from South African Snake Farm. Tissue culture dishes were obtained from Becton Dickinson Labware, Oxnard, Calif., and tissue culture plates (model FB 16-24) were obtained from Linbro Scientific, Hamden, Conn.

**Preparation and culture of cells.** Cells were obtained from tumors transplanted serially on the chorioallantoic membranes of 10-day-old chicken embryos; the tumors were excised, minced, and dissociated into single-cell suspensions as previously described (33). They were grown routinely in Dulbecco medium supplemented with 10% fetal bovine serum. In addition to HEp-3 cells, the following lines were used: Colo 16 (30), Colo 227 (a squamous cell carcinoma which appeared in an old burn scar), M8342 melanoma, and Bowes melanoma, all kindly provided by G. Moore; a bladder carcinoma and a hypernephroma (49), provided by E. L. Wilson; and HeLa cells obtained from W. D. Schleuning.

**Treatment of cells with DMSO.** HEp-3 and other cell lines were plated at $4 \times 10^5$ to $7 \times 10^5$ cells per dish (60 mm) in medium with 10% serum. After the cells attached, the medium was replaced by fresh medium containing 5% serum and the indicated concentration of DMSO. To establish the onset of morphological change, the cells were examined microscopically every few hours. PA production was determined by measuring the enzyme activity in aliquots of growth medium by the radioactive fibrin plate method (45, 46). In some experiments, the effect of DMSO on cell division was monitored by trypsinizing duplicates of control and DMSO-treated cultures daily for 4 days and counting the cells in an hemacytometer.

**RNA extraction.** The RNA from early passages of control and DMSO-treated HEp-3 cells was extracted as described previously (1). Briefly, the cells were scraped, pelleted, suspended in buffer, and lysed by Nonidet P-40. The cytoplasmic extracts were digested with proteinase K, extracted with phenol-chloroform-isoamylalcohol, precipitated with ethanol, and suspended in water. The poly(A)$^+$ RNA was purified by batch-wise elution from oligo(dT)-cellulose fines (31). The RNAs were translated in vitro with wheat germ extracts (13).

**PA production by oocytes injected with RNA.** Oocyte injections and measurements of PA in the oocyte extracts were performed exactly as previously described (1, 16, 29).

**Northern blot hybridization.** Northern blots, prepared as described previously (1), were hybridized to pDB15, a mouse uPA cDNA probe (2). To allow for cross-species hybridization, the hybridization was performed at 58°C and the washings were performed at room temperature (8).

**PA inhibitor assay.** The PA inhibitors secreted by HEp-3 cells were assayed as described previously (47). Briefly, a constant amount of $^{125}I$-labeled uPA was incubated for 1 h at 4°C with the indicated volumes of conditioned medium from control and DMSO-treated cells. The mixtures were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

## RESULTS

**Effect of DMSO on PA.** HEp-3 tumor extracts contain high uPA activity (33). When explanted, confluent primary cell cultures of this tumor produce high levels of uPA for several days (34). Under these conditions, the amount of uPA recovered from the medium over a period of 24 h exceeded by about 20-fold the amount associated with cells at any given time (Fig. 1). Within 6 h after the addition of DMSO, the cell-associated uPA decreased to 25 to 30% of the control value and remained at this level for the duration of treatment (Fig. 2); the secreted uPA was reduced to 50% of the control level after 6 h and became undetectable after 24 to 48 h of treatment.

To test whether DMSO affects all uPA-producing cells to the same extent, DMSO-treated and control cells seeded at low density were incubated under a casein-plasminogen-agar

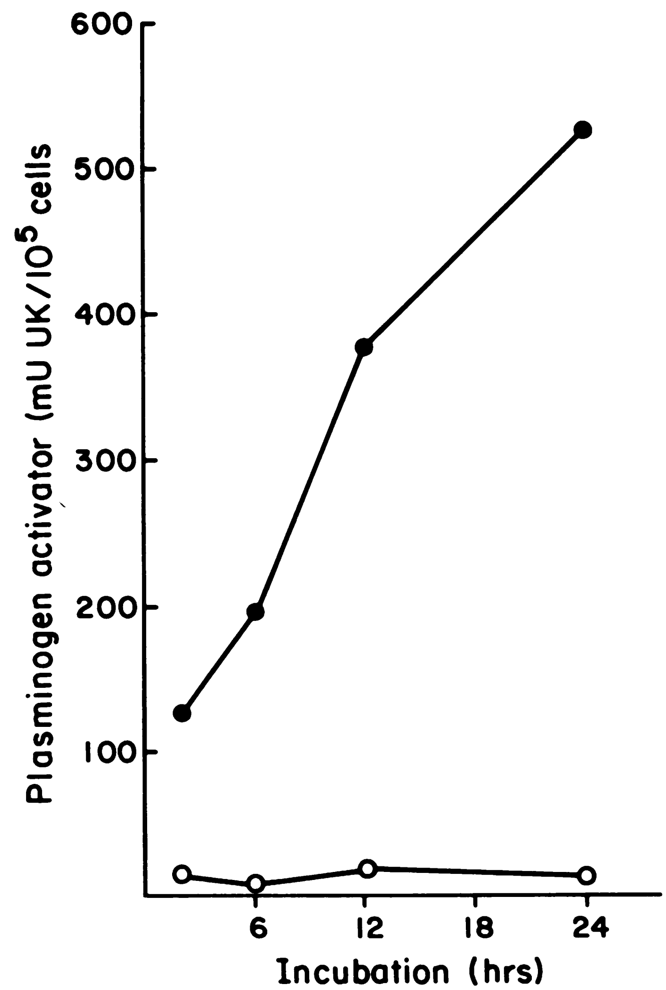


FIG. 1. Kinetics of uPA production by primary cultures of HEp-3 cells. Primary cultures of HEp-3 cells were seeded at $3 \times 10^5$ per 35-mm dish. After an overnight incubation, the medium was replaced with fresh medium containing 5% serum. At the indicated times, media and cells were collected and analyzed for uPA content as described in Materials and Methods. Symbols: ●, secreted uPA; ○, cell-associated uPA.

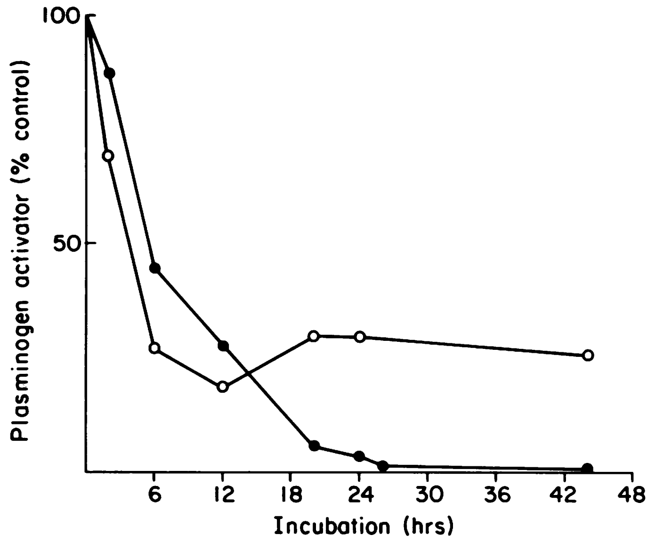


FIG. 2. Effect of DMSO on uPA production by HEp-3 cells. Primary cultures of HEp-3 cells were seeded at 7 × $10^5$ cells per 60-mm dish. After an overnight incubation, the medium was replaced with fresh medium containing 5% serum with or without 1.4% DMSO. At the indicated times, conditioned media and cells were collected and analyzed for uPA as described in Materials and Methods; uPA content of DMSO-treated cells is expressed as a percentage of the uPA content of control cultures. Each experimental point is the average of four dishes from two independent experiments. Symbols: ●, secreted uPA; ○, cell-associated uPA).

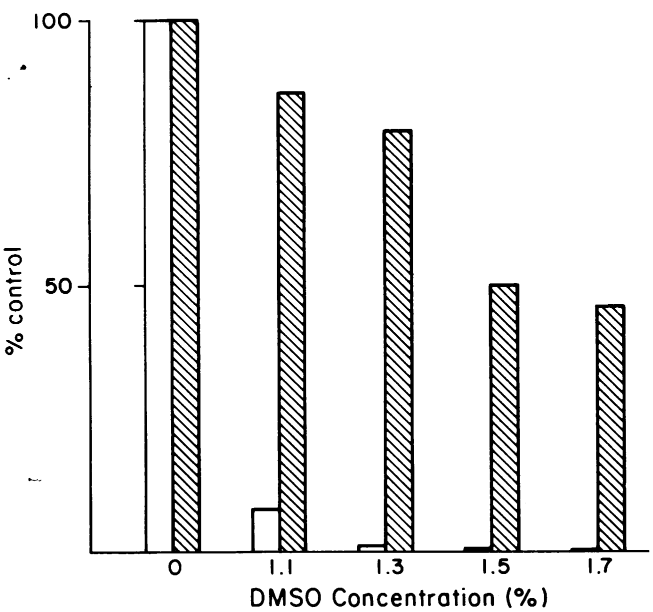


FIG. 3. Effect of DMSO concentration on uPA production and on cell growth. Primary HEp-3 cells were seeded at 7 × $10^5$ per 60-mm dish. After an overnight incubation, the medium was replaced with fresh medium supplemented with 5% serum and increasing (1.1 to 1.7%) concentrations of DMSO. Medium was changed daily on all cultures. Conditioned medium collected between 72 and 96 h was used to determine uPA activity. Cells were trypsinized and counted; 2.8 × $10^6$ cells per dish were recovered from control cultures. Each experimental point (expressed as a percentage of control values) is an average of two dishes. Symbols: ☐, uPA activity; ▨, number of cells.

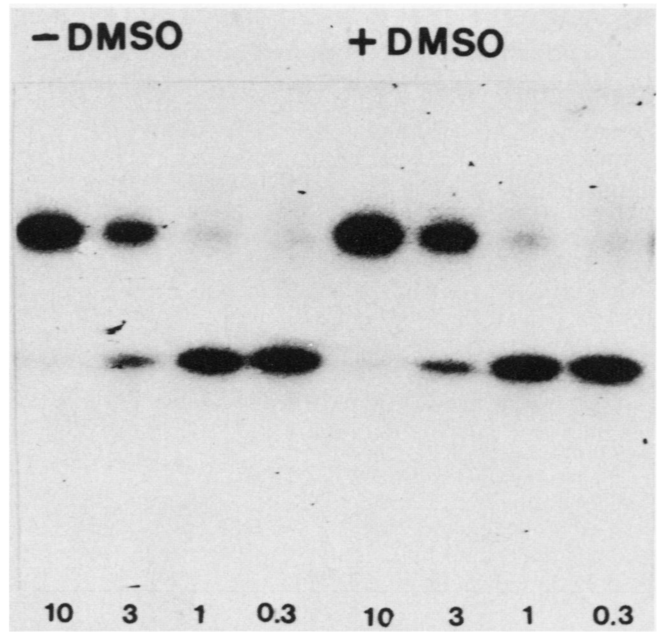


FIG. 4. Effect of DMSO on PA inhibitor production by HEp-3 cells. Early passages of HEp-3 cells were incubated for 24 h in medium containing 5% fetal bovine serum with (+DMSO) or without (−DMSO) 1.4% DMSO. Conditioned media were collected and serially diluted in phosphate-buffered saline containing 1 mg of acid-treated bovine serum albumin per ml. The samples (volumes in microliters are indicated under each lane) were incubated with 1.35 ng of $^{125}$I-labeled uPA (5 μl) and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. From the left side, the first four lanes contain control samples; the remaining four contain DMSO-treated samples.

overlay (17). In this assay, each PA-producing cell formed a lytic zone in the opaque casein layer. After 7 h, about 40% of the control and none of the DMSO-treated cells were surrounded by lytic zones (data not shown).

**Mechanism of DMSO-induced inhibition of uPA production.** The effect of increasing DMSO concentrations on HEp-3 cell growth and uPA production is shown in Fig. 3. The two dose-response curves are very different: DMSO concentrations that had only a minor effect on cell growth (1.1 to 1.3%) strongly inhibited uPA production, suggesting that this effect is not the result of an overall inhibition of macromolecular synthesis.

Several events may lead to a rapid decrease in PA activity: PA or plasmin might be inactivated by the solvent; DMSO might induce the production of PA inhibitors, or it could reduce the concentration of uPA mRNA or interfere with its translation.

The first possibility was excluded by mixing DMSO with conditioned medium from untreated HEp-3 cells; no apparent loss of catalytic activity was observed. To examine the second possibility, we took advantage of the capacity of PA inhibitors to form covalent complexes with uPA (47). Purified, $^{125}$I-labeled uPA was incubated with the indicated volumes of conditioned medium from control and DMSO-treated cultures. The uPA-inhibitor complexes were separated from the unreacted enzyme by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and revealed by autoradiography (Fig. 4). The relative amount of complexed uPA versus free uPA increased proportionally with the increase in the volume of conditioned medium added to the reaction mixture. The pattern of complex formation was

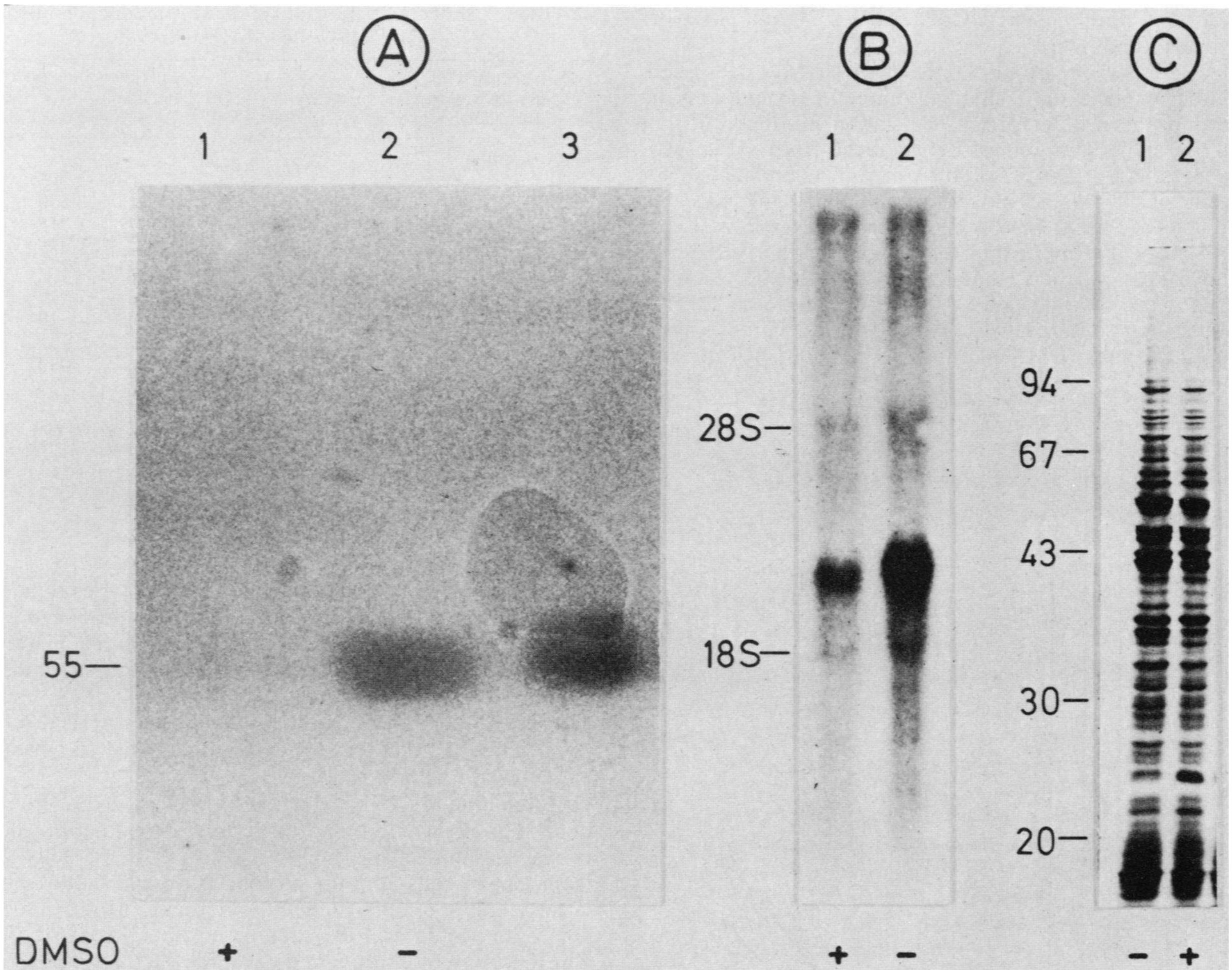


FIG. 5. Effect of DMSO on the uPA mRNA content of HEp-3 cells. Early passages of HEp-3 cells were incubated for 24 h in medium containing 5% fetal bovine serum with or without 1.4% DMSO. Total cytoplasmic RNA was isolated and analyzed as described below for each panel. (A) Concentration of functional uPA mRNA. *X. laevis* oocytes were injected with 180 ng of cytoplasmic RNA from DMSO-treated cells (lane 1) or control cells (lane 2). Pools of 10 oocytes were incubated for 24 h, extracted, and analyzed by zymography as described previously (1). Lane 3, Purified uPA standard. (B) uPA-mRNA abundance. Cytoplasmic RNAs (10 μg per lane) from DMSO-treated (lane 1) and control (lane 2) cells were electrophoresed in a 1.2% agarose gel and transferred to nitrocellulose. RNAs electrophoresed in adjacent lanes were stained with ethidium bromide to determine the position of 18S and 28S RNAs. The filter was hybridized under nonstringent conditions (8) to a mouse uPA cDNA probe (2) and autoradiographed. (C) Profile of total translation products. Cytoplasmic RNAs (8 μg) from control (lane 1) and DMSO-treated (lane 2) cells were translated in presence of [$^{35}$S]methionine in a wheat germ extract (13). The translation products were electrophoresed under reducing conditions in a 9% polyacrylamide gel and revealed by fluorography (1). The migration of standard proteins (Pharmacia low-molecular-weight kit) was determined by Coomassie blue staining, and their molecular weights are given in thousands.

identical in conditioned media from control and DMSO-treated cells, indicating that they both contained equivalent amounts of free uPA inhibitors. Several different PA inhibitors have been identified in culture media of mammalian cells. By using monospecific antibodies that discriminate between a PA inhibitor isolated from placenta (T. C. Wun and E. Reich, J. Biol. Chem., in press) and one secreted by endothelial cells (24), we found that both types were present in the culture medium of HEp-3 cells and that DMSO did not alter their relative proportion (J.-D. Vassalli and D. Belin, unpublished observations). Taken together, these results indicate that enhanced production of PA inhibitors does not account for the effect of DMSO on HEp-3 cells.

To determine the effect of DMSO on uPA mRNA content, we measured the relative concentration of functional uPA mRNA by analysis of the translation products of RNA-injected *X. laevis* oocytes (Fig. 5A). Oocytes injected with RNA from control cultures (Fig. 5A, lane 2) synthesized a PA that comigrated with a purified human uPA standard (lane 3). Only small amounts of uPA were recovered from oocytes injected with RNA from DMSO-treated cells (Fig. 5, lane 1). Thus, the concentration of functional uPA mRNA is significantly reduced in DMSO-treated cells.

We used a murine uPA cDNA probe (1, 2) to compare the uPA mRNA contents of control and DMSO-treated cells in Northern blots (Fig. 5B). A densitometric analysis of the autoradiogram indicates that DMSO treatment reduced the uPA-mRNA level to about 20% of that found in control cells. Similar results were obtained with poly(A)$^+$ RNA (data not shown). We also analyzed the kinetics of uPA mRNA decrease upon addition of DMSO to HEp-3 cells: no difference was detectable in the first 2 h; after 4 h, the uPA mRNA level was reduced twofold; a maximal decrease, to 20% of the control uPA mRNA content, was observed at 13 h; and no further decrease was evident after 24 and 48 h of treatment (not shown).

RNAs from control and DMSO-treated free cells were translated with the same efficiency in cell extracts, and the electrophoretic profiles of the translation products did not reveal any major differences between the RNAs (Fig. 5C). Thus, the effect of DMSO on uPA mRNA abundance is not the result of decreased contents of all cellular mRNAs.

**The effect of DMSO on uPA production is reversible.** HEp-3 cultures were first incubated for 3 weeks, either in control medium or in medium containing 1.4% DMSO. Control cultures were maintained in medium without DMSO; DMSO-treated cultures were either transferred to control medium or kept in DMSO-containing medium. PA activity

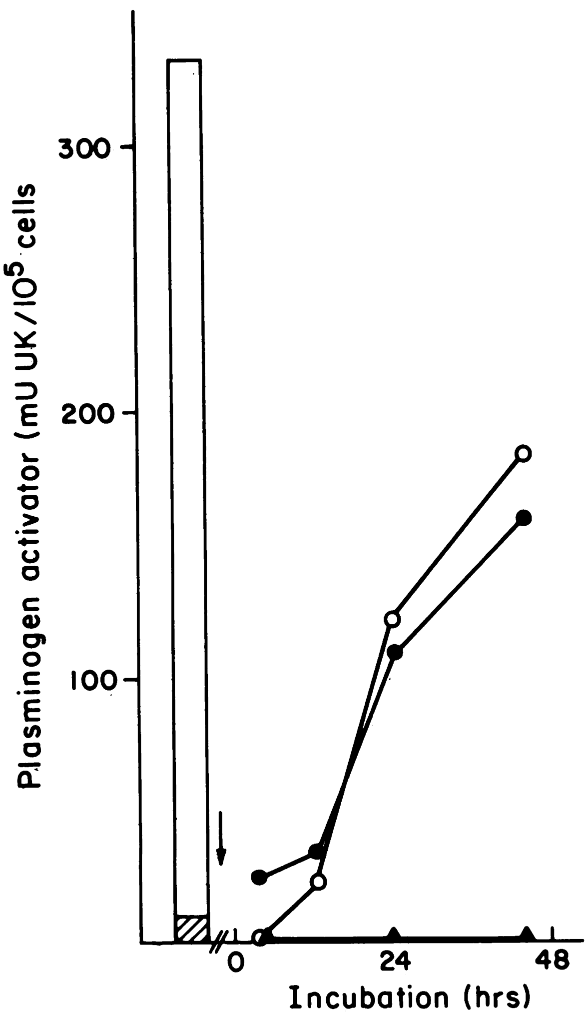


FIG. 6. Reversibility of the DMSO effect on uPA production. HEp-3 cells were passaged for 3 weeks (six passages) in medium containing 10% serum with or without 1.4% DMSO. The cells were then plated at $3 \times 10^5$ cells per 60-mm dish in the same medium. After an overnight incubation, the uPA content of conditioned medium of control (□) and DMSO-treated (▨) cells was determined. The cultures were divided into three groups: cells pretreated with DMSO and maintained in DMSO-containing medium (▲), cells pretreated with DMSO and transferred to DMSO-free medium (○), and cells kept continuously in DMSO-free medium (●). Aliquots of the culture media were collected at the indicated times and assayed for uPA activity. Each experimental point is the average of results from two dishes.

TABLE 1. Effect of cholera toxin on uPA production in control and DMSO-treated HEp-3 cells[a]

| Treatment group, cholera toxin concn (M) added | uPA level in conditioned medium (mU of urokinase activity per $10^6$ cells) |
|---|---|
| Control cells | |
| 0 | 2,505 |
| $10^{-11}$ | 2,350 |
| $10^{-10}$ | 1,715 |
| $10^{-9}$ | 1,485 |
| DMSO-treated cells | |
| 0 | 10 |
| $10^{-11}$ | 135 |
| $10^{-10}$ | 430 |
| $10^{-9}$ | 200 |

[a] Primary HEp-3 cells were seeded at $7 \times 10^5$ cells per 60-mm dish and incubated overnight in medium supplemented with 10% serum. The cultures were incubated first for 24 h with fresh medium containing 5% serum with or without DMSO and then for 48 h with the same medium containing the indicated concentrations of cholera toxin.

was measured at different times in all three types of cultures (Fig. 6). In less than 12 h, the uPA activity produced by the cells which had been preincubated in DMSO reached that of control cells. As expected, uPA activity remained undetectable in cultures continuously maintained in DMSO. A similar kinetics of uPA production upon removal of DMSO was observed with cells which had been exposed to DMSO for either 1 day or for 3 weeks.

**Modulation of uPA production by cholera toxin.** We previously established that the response to modulators of uPA production in primary, highly malignant HEp-3 cells differs from that of cells kept for prolonged time in culture. In the latter, cholera toxin significantly increased uPA production, whereas no effect was detected with primary cultures (34). It was therefore of interest to determine whether cholera toxin also stimulates uPA production in DMSO-treated cells. Control cells and cells pretreated with 1.2% DMSO were exposed to increasing concentrations of cholera toxin, and the conditioned media were tested for uPA activity (Table 1). As shown previously, cholera toxin did not stimulate uPA production in primary cultures of HEp-3 cells; a slight inhibition was observed with the highest concentrations. In contrast, the DMSO-treated cells responded to cholera toxin with a 20- to 40-fold increase in uPA production, rising to a maximum of about 20% of the level of uPA secreted by control cells.

**Effect of DMSO on cell morphology.** Primary cultures of HEp-3 tumors consisted of heterogeneous, highly refractile, loosely attached cells which formed disorganized layers, crisscrossed by long cellular projections (Fig. 7A). The cells did not form an organized pattern nor did they fill the intercellular spaces, even at confluency. Upon prolonged culture in vitro, the cellular projections disappeared, and the cells became less refractile, and formed homogenous, mosaic-like layers of cells which were firmly attached to the substratum (Fig. 7B). Similar changes occurred upon addition of DMSO to primary HEp-3 cells: this altered morphology was first detectable 24 h after the addition of DMSO and was fully established after 48 h (Fig. 7C).

**Effect of DMSO on PA production in tumor cells of different origins.** Mammalian cells produce at least two types of PAs, which are the products of separate genes (39, 48) and whose expression is under tissue-specific hormonal control. Human tumors of different origins produce either uPA, the type of

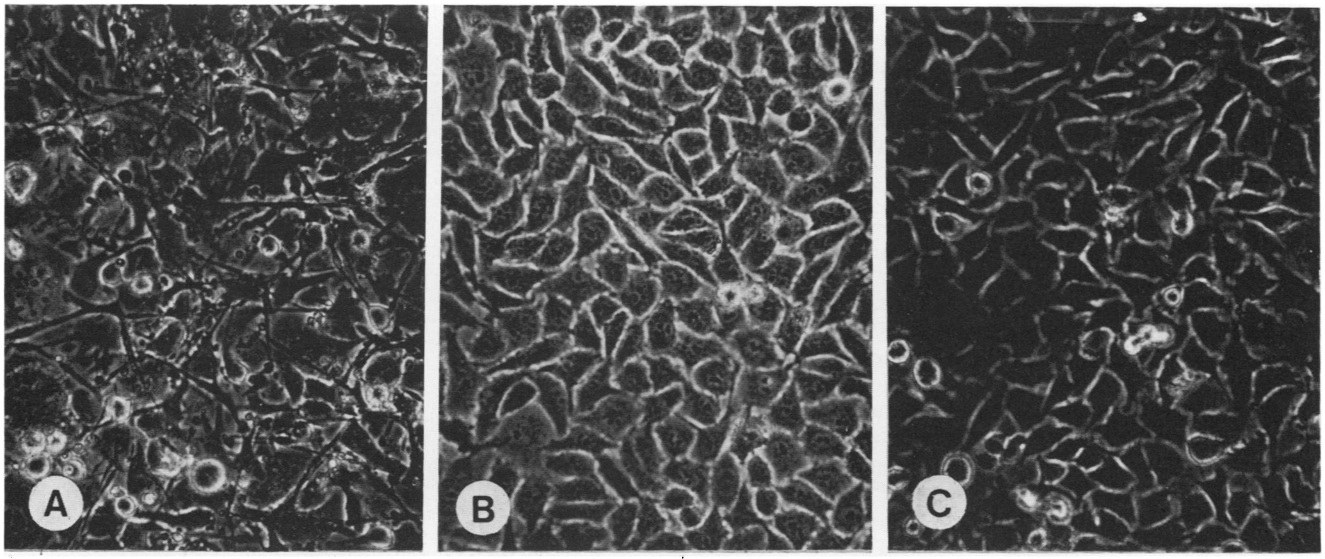

FIG. 7. Effect of DMSO on the morphology of HEp-3 cells. (A) Primary cultures of HEp-3 tumor cells. (B) Culture of nonmalignant HEp-3 cells after 29 passages in vitro. (C) The same cells as in panel A incubated for 72 h in the presence of 1.4% DMSO. The cells were fixed with 2.5% glutaraldehyde and photographed under phase contrast. Magnification, ×200.

PA produced by HEp-3 cells, or the tissue-type PA (tPA). We examined the effect of DMSO on PA production in eight different tumor cell lines (Table 2). Only uPA-producing cells were inhibited by DMSO, and the inhibition appeared to be most pronounced in lines obtained from squamous cell carcinomas. In contrast, the enzyme activity in lines producing tPA was either only slightly inhibited or stimulated by DMSO.

## DISCUSSION

Results obtained in a number of tumor systems, in addition to our previous observations with HEp-3 cells, indicate that malignancy may behave as a conditional phenotype (35). Thus, the identification of factors which regulate the expression of the malignant phenotype should be of great importance, both theoretically and clinically. The transition of HEp-3 cells from a malignant to a nonmalignant state, which takes place in culture, is accompanied by a number of changes, including a reduction in uPA synthesis (34). We show here that DMSO treatment of HEp-3 cells has a similar but much more rapid effect on uPA production.

TABLE 2. Effect of DMSO on uPA- and tPA-producing tumor cells lines[a]

| PA type, cell line | Tumor type | PA (% control) for cells treated with (1.2%) DMSO | PA (% control) for cells treated with (1.4%) DMSO |
|---|---|---|---|
| uPA | | | |
| HEp-3 | Squamous | 10 | 3 |
| Colo 16 | Squamous | 19 | 4 |
| Colo 227 | Squamous | 6 | 3 |
| UCT-BC | Bladder | 18 | 11 |
| UCT-HN | Hypernephroma | 18 | 15 |
| tPA | | | |
| Bowes | Melanoma | 175 | 157 |
| M8342 | Melanoma | 72 | 78 |
| HeLa | Cervical | 277 | 229 |

[a] The different tumor cell lines were plated at $4 \times 10^5$ to $7 \times 10^5$ cells per 60-mm dish in medium supplemented with 10% heat-inactivated fetal bovine serum and incubated overnight. Cultures were divided into three groups: a control group incubated with medium plus 5% serum, and two groups incubated in media (plus 5% serum) containing 1.2 and 1.4% DMSO, respectively. Secreted PA activity was determined after 48 h. Results are the averages of duplicate cultures and duplicate PA determinations. The largest difference between the duplicate cultures was <25%, and the largest difference between the PA determinations was <10%. Tumor types used were squamous carcinoma, bladder carcinoma, hypernephroma, and cervical carcinoma.

Our results clearly indicate that a reduction in the functional concentration and chemical abundance of uPA mRNA can at least partially account for the reduced level of uPA production by DMSO-treated cells. This effect can be explained either by reduced uPA gene transcription or by altered uPA-mRNA stability (3, 20, 22, 26).

An apparent discrepancy exists between the fivefold reduction in uPA mRNA level and the 20- to 40-fold reduction in total uPA activity observed. Several factors could account for this discrepancy. The translation efficiency of uPA mRNA may be specifically reduced. Alternatively, uPA activity recovered from conditioned medium may not accurately reflect uPA synthesis. Several factors have been shown to affect estimates of uPA protein levels based on uPA fibrinolytic assays. First, the cosecretion of PA inhibitors and uPA, in its unreactive proenzyme form (50), has been documented (47). Under our culture conditions, a significant portion of the pro-uPA secreted by control HEp-3 cells could have been activated; it would have reacted with the PA inhibitors, whose concentrations vastly exceed that of uPA. Second, uPA may not be stable in the culture medium and could be readsorbed by the cells, as shown for tPA in cocultures of human melanoma cells and fibroblasts (18). Such interactions might reduce the amount of measurable, secreted uPA and contribute to the observed discrepancy in the inhibition of uPA mRNA and the enzyme activity by DMSO.

The effect of DMSO has been studied extensively in Friend leukemia cells (7, 15, 27, 41), and in other tumor systems (11, 19, 21, 40). Although its mode of action remains unclear, it has been suggested that DMSO works by initiating transcription of silent genes, either by interfering with

DNA methylation (7) or by relaxing the DNA and making it more accessible to polymerase(s) (41). In most of the tumors or tumor cell lines, these silent genes are thought to be responsible for differentiated functions in the tissue of origin, and their induction would restore the original transcriptional program. This differentiation has been associated with a loss of tumorigenicity in several instances (23, 40).

A decreased steady-state level of uPA mRNA is first detectable 4 h after the addition of DMSO to HEp-3 cells. This delayed effect might thus be the consequence of a silent gene(s) activation.

The effect of DMSO on uPA appears to be relatively specific: cell growth is only slightly inhibited, and most species of the abundant mRNA do not seem to be affected. A similar result has been reported with Friend leukemia cells (27); by using two-dimensional polyacrylamide gels in which 400 in vitro-translated proteins were resolved, the authors found that only 3 proteins were different in the DMSO-treated cells. Finally, the inhibition of PA production by DMSO appears to be restricted to uPA-producing cells.

Two additional properties which are acquired by HEp-3 cells upon prolonged culture in vitro also could be induced by DMSO, but much more rapidly. Within 24 to 48 h of treatment, the morphology of each individual HEp-3 cell as well as the general appearance of the culture changed; the disarrayed spatial orientation, characteristic of malignant cells, was replaced by a new pattern formed by cells arranged in mosaic-like monolayers. These flattened cells also acquired the ability to respond to cholera toxin with an enhanced uPA production and a partial restoration of the original morphology. The DMSO-induced alteration in morphology and in response to cholera toxin are secondary to the inhibition of uPA production.

The reversible transition between the malignant and the nonmalignant state of HEp-3 cells can be induced slowly and gradually by changing their growth conditions (34, 35). Addition or removal of DMSO induces a similar set of changes with much more rapid kinetics. Therefore, it can be viewed as a compressed version of the events that take place during the growth of HEp-3 cells in vitro.


## ACKNOWLEDGMENTS

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